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Review

Control of *Listeria monocytogenes* growth by bacteriocin-producing starter cultures in the manufacturing of dry fermented sausage

Renata Ernlund Freitas de Macedo[1], Lye Miyague[1], Leandro Batista Costa[2] and Fernando Bittencourt Luciano[1]*

[1]School of Agricultural Sciences and Veterinary Medicine, Pontifícia Universidade Católica do Paraná, BR 376 Km 14, São José dos Pinhais, PR, 83010-500, Brazil.
[2]Department of Agricultural and Environmental Sciences, Universidade Estadual de Santa Cruz, Rod. Ilhéus/Itabuna Km 16, Ilhéus, BA, 45662-900, Brazil.

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The production of dry fermented sausages started as a home-made process, but large-scale industrial manufacture of these products has encountered some safety issues. Although these products present extended shelf-life and offer various hurdles against the growth of undesired organisms, pathogens such as *Listeria monocytogenes* were found to survive. Bacteriocins have been proposed to act as an extra barrier against the growth of Gram-positive bacteria, including *L. monocytogenes*. These antimicrobials are synthesized by some lactic acid bacteria (LAB), which are responsible for the fermentation of dry sausages. Many bacteriocins produced by LAB showed ability to inhibit *L. monocytogenes* growth (e.g. enterocin, pediocin and sakacin). However, the levels of bacteriocins needed to produce this effect in food systems are much higher than those necessary *in vitro*. Use of bacteriocinogenic starter cultures have shown promising anti-listerial activity in dry sausage, since the antimicrobials are produced *in situ* confering a better distribution and sustained synthesis throughout the fermentation period. This manuscript reviews the technological challenges of bacteriocin application in dry fermented sausage and the recent findings on using bacteriocinogenic autochtonous LAB to improve safety towards *L. monocytogenes*.

Key words: Fermented sausages, *Listeria monocytogenes*, bacteriocins.

INTRODUCTION

Dry fermented meat products have been consumed all over the world for centuries and are characterized by the growth of microorganisms that produce lactic acid, which drops the pH and generates a distinctive flavor (Getty et al., 2000). Peculiarities of process, recipes and fermenting microorganisms are as diverse as the number of countries where these products are manufactured (Lebert et al., 2007). Generally, dry fermented sausages are composed of salt, curing salt, sugar, spices, herbs, lactic acid bacteria (LAB) and lean meat and fat. Therefore, LAB growth and metabolite production during the sausage ripening will depend on the product composition and process parameters (temperature, humidity, use of smoke or not) (Drosinos et al., 2006). In addition, knowledge about the characteristics of the LAB used for manufacturing dry sausages is of paramount importance to achieve the desired sensorial quality and also to enhance food safety. Traditionally, the fermentation process of dry sausages was as a result

*Corresponding author. E-mail: fernando.luciano@pucpr.br. Tel: +55 41 3299-4334. Fax: +55 41 3299-4423.

of intrinsic LAB growth. In other words, the bacteria were pre-existent in the meat and no external inoculum was used. More recently, with the advance of molecular methods, these bacteria have been identified and a variety of them were made commercially available. Their composition depends on the type of final product desired, but usually comprises a combination of LAB and Gram-positive catalase positive cocci (Aymerich et al., 2003). Among the LAB, *Lactobacillus sakei*, *Lactobacillus curvatus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Pediococcus pentosaceus* and *Pediococcus acidilactici* are the most commonly used for production of dry fermented sausages (Ammor and Mayo, 2007). The utilization of Gram-positive catalase positive cocci is necessary due to their ability to degrade the H_2O_2 produced by the LAB (avoiding color issues and rancidification) and for reducing nitrate in nitrite (Työppönen et al., 2003a). *Staphylococcus xylosus*, *Staphylococcus carnosus*, *Staphylococcus equorum*, *Staphylococcus succinus* and *Staphylococcus saprophyticus* are the most frequently found for dry fermented sausage preparation (Drosinos et al., 2007; Mauriello et al., 2004).

Fermented sausages generally offer barriers, known as “hurdles”, for the growth of opportunistic pathogenic bacteria. These hurdles comprise the low pH, high salt level, presence of organic acids and nitrite, low a_w, competition with the resident microbiota, addition of spices, herbs and smoke (Työppönen et al., 2003a). Even though, pathogens like *Listeria monocytogenes* and *Escherichia coli* O157:H7 are tolerant to high levels of salt and low pH, they were found to persist and eventually grow in dry fermented sausages (Clavero and Beuchat, 1996; Hinkens et al., 1996; Talon et al., 2008). The control of *L. monocytogenes* has been found to be extremely difficult due to its ubiquitous presence in meat processing plants and its psychrotrophic nature. Salvat et al. (1995) found that 68% of the swab samples collected from 7 different meat-processing plants were positive for *L. monocytogenes* in France. In addition, 16% of dry fermented sausages were found positive for *L. monocytogenes* in a Turkish study (Çon et al., 2001) and the occurrence of the pathogen was found in 12 to 16% of the industrial fermented foods produced in Europe (Ananou et al., 2005). The main source of *L. monocytogenes* is usually contaminated raw meat, and its prevalence is more pronounced in sausages maintained for short ripening periods (e.g. German Mettwurst) (Leroy et al., 2005). Strains from the serogroup 1 are the most common in food, but outbreaks are usually related with *L. monocytogenes* strains of the serogroup 4 (Buncic et al., 2001). It is also important to mention that this pathogen is usually harmless to normal individuals, but presents a serious hazard to infants, elderly, immunocompromised and pregnant people (Farber and Peterkin, 1991).

Some lactic acid bacteria produce peptides/proteins with antibacterial activity called bacteriocins, and the use of these proteinaceous compounds has been suggested as an extra “hurdle” against the growth of pathogenic bacteria (Ananou et al., 2005; Drosinos et al., 2006; Kuleasan and Çakmakçi, 2002). Diverse studies have shown the bactericidal or bacteriostatic effect of bacteriocins against *L. monocytogenes in vitro* and in meat products (Benkerroum et al., 2005; Hugas et al., 1995). However, the doses of bacteriocins needed to inhibit *Listeria* outgrowth in meat products were reported to be much higher than those found *in vitro* (Gálvez et al., 2007; Hartmann et al., 2011; Zhang et al., 2010). Inactivation by proteases, reaction with intrinsic components of meat and limited diffusion are some of the theories trying to explain this fact (Leroy and de Vuyst, 1999). In addition, some studies have reported no significant difference on listerial growth when the pathogen grew concomitant with either a bacteriocinogenic LAB or a non-bacteriocinogenic strain (Katla et al., 2001; Leroy et al., 2005).

More recently, the identification and utilization of specific indigenous bacteriocinogenic LAB as starter cultures for the production of dry fermented sausages has been proposed (Villani et al., 2007). Theoretically, these strains could enhance the safety of these products since they are highly adapted to the environment, which increases the chance of eliminating possible bacterial contaminants from the food system (Talon et al., 2008). This article will review the efforts to avoid the growth of *L. monocytogenes* in dry fermented sausages, emphasizing the utilization of bactericionogenic LAB as starter cultures of these products.

FACTORS INFLUENCING THE GROWTH OF PATHOGENS IN DRY SAUSAGE

The basic formula to produce dry fermented sausages consists of the mixture of pork and beef meat together with pork fat. Other common ingredients in the composition of a general dry fermented sausage are salt, nitrite, nitrate, sugar, ascorbate, spices and the starter culture (Työppönen et al., 2003a). Many of these ingredients work as “hurdles” against undesirable microorganisms (Figure 1). First, nitrite is a well-known antibacterial agent and its production is maintained throughout the fermentation process by the starter culture reduction of nitrate (Työppönen et al., 2003b). High levels of salt also inhibit the growth of various saprophytic and pathogenic bacteria. Moreover, many of the spices used in the manufacture of dry sausages contain essential oils with recognized antimicrobial activity (eg. pepper, mustard and garlic) (Holley and Patel, 2005). In addition, the starter culture offers more than one barrier against the growth of other bacteria. They are tough competitors

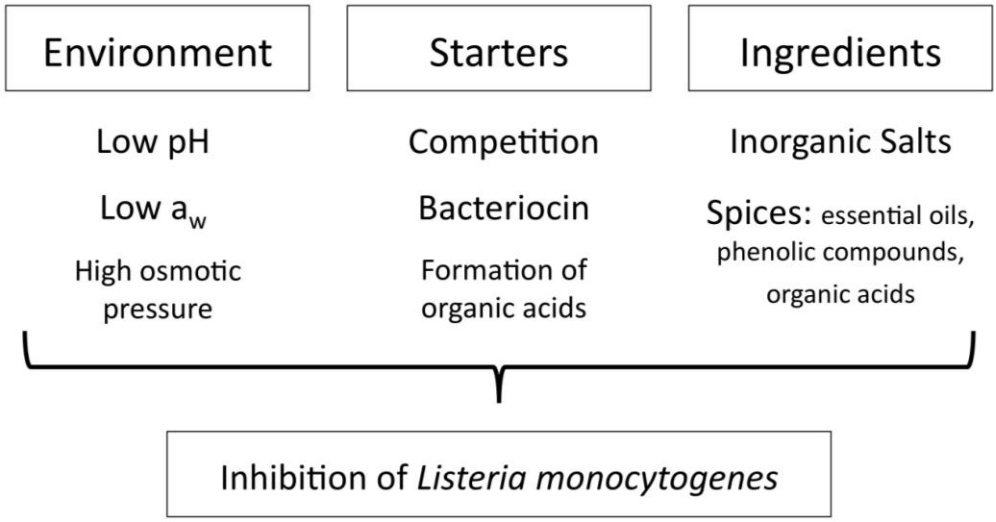

Figure 1. Hurdles present in dry fermented sausage against the growth of *L. monocytogenes.*

for the utilization of nutrients, have the ability to produce organic acids (lactic acid, acetic acid) and cause rapid drop of the sausage pH. At low pH, organic acids are found in their molecular form, which is able to cross the bacterial cytoplasmic membrane. Then, these acids are dissociated in the bacterial cytoplasm, reducing its pH and altering the electrochemical proton gradient leading to cellular death (Podolak et al., 1996). The low pH itself also inhibits the growth of many bacteria (Gálvez et al., 2007).

Additionally, lactic acid bacteria produce other bactericidal/bacteriostatic compounds such as hydrogen peroxide, carbon dioxide, diacetyl and bacteriocins (Ammor et al., 2006). Hydrogen peroxide is a precursor of free radicals, which are reactive compounds that can damage cellular structures such as the cytoplasmic membrane and DNA (Morris, 1976). High level of carbon dioxide is well known to inhibit the growth of diverse bacteria (Lindgren and Dobrogosz, 1990) and diacetyl decreases arginine utilization by the cell, affecting the growth of some Gram-negative bacteria (Jay, 1982).

Some strains of LAB used as starter cultures have the capacity to produce bacteriocins. These are peptides or proteins with bactericidal or bacteriostatic effect against other bacteria (Alves et al., 2006; Benkerroum et al., 2005; Goff et al., 1996). The mechanism of action of how the bacteriocins affect the bacterial cells is still not fully understood, but they were found to form pores in the cytoplasmic membrane, interfere with cell wall synthesis and promote cell wall lysis, causing the release of metabolites and collapse of the proton motive force (Montville et al., 1995). These antimicrobials were found to be more effective against Gram-positive bacteria. The reason for this fact was related with the presence of an outer membrane in Gram-negatives, which exert a function of protective shield, avoiding the interaction of bacteriocins with the cytoplasmic membrane and cell wall (Työppönen et al., 2003a). Helander et al. (1997) have suggested the utilization of an edible permeabilizing agent that could help these bacteriocins to transpose the outer membrane of Gram-negatives and reach their supposed action target.

To date, “nisin”, synthesized by *Lactococcus lactis*, is the most studied bacteriocin and the only one approved as a food additive (Al-Holy et al., 2012; Hansen, 1994). However, numerous other bacteria were described to produce bacteriocins such as *Lactobacillus sakei* (Leroy and de Vuyst, 1999), *Enterococcus faecalis* and *Enterococcus faecium* (Ananou et al., 2005; Herranz et al., 2001), *Lactobacillus reuteri* (Kuleasan and Çakmakçi, 2002), *Lactobacillus buchneri* (Minor-Pérez et al., 2005), *P. acidilactici* (Nieto-Lozano et al., 2005) and *P. pentosaceus* (Todorov and Dicks, 2005).

The hurdles presented in dry fermented sausages are able to prevent the growth of common food pathogens such as *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium botulinum* (Työppönen et al., 2003a). However, *L. monocytogenes* (Faber and Peterkin, 1991) and *E. coli* O157:H7 (Hinkens et al., 1996) are able to persist in this harsh environment due to their ability to resist stresses caused by low pH and high salt content. Both bacteria have very low infectious doses and have caused food outbreaks related with the consumption of meat products. In 1994, *E. coli* O157:H7 derived from dry fermented sausages was responsible for 18 people been ill in the United States. This fact led the American and Canadian health authorities to demand a minimum of 5 Log CFU/g reduction of *E. coli* O157:H7 during the dry

fermented sausage manufacturing process (Hinkens et al., 1996; Palanichamy et al., 2008).

No outbreak was correlated with the presence of *L. monocytogenes* in dry fermented sausages so far (Leroy and de Vuyst, 1999), but the ability of this pathogen to survive in the food matrix has been proven and it represents a potential hazard to humans (Gálvez et al., 2007). In addition, the United States has a zero-tolerance policy regarding the presence of *L. monocytogenes* in ready-to-eat foods (Chen et al., 2003). This has led many exporting countries, such as Spain, Italy and Germany, to apply expensive techniques of food hygienization (high pressure processing) before sending fermented/raw meat products to the US. Many scientists have proposed the utilization of isolated bacteriocins or bacteriocinogenic starter cultures to combat this pathogen (Ammor and Mayo, 2007; Çon et al., 2001; Leroy et al., 2005; Benkerroum et al., 2005; Alves et al., 2006).

There are other metabolites produced by bacteria that have shown antimicrobial activity, and among these substances, reuterin (β-hydroxypropinaldehyde) - produced by *L. reuteri* is one of the most studied (Arqués et al., 2008; El-Ziney and Jakobsen, 2009; Rasch, 2002; Shaefer et al., 2010). However, there are very few studies in the literature using purified reuterin to eliminate *L. monocytogenes* in sausage. The effect of the aldehyde on the growth of *L. monocytogenes* and *Salmonella* spp. on the surface of traditional Turkish sausages was subject of study (Kuelasan and Çakmakçi, 2002). The authors showed that the aldehyde could significantly reduce the population of *L. monocytogenes* after 7 days, but it did not eliminate the pathogen. In addition, no effect on *Salmonella* spp. growth was observed throughout the study. Although reuterin seems to be a good antilisterial agent *in vitro* and for application in dairy products (El-Ziney et al., 1998; El-Ziney and Jakobsen, 2009), its use to eliminate this foodborne pathogen in sausages seems to be very limited.

Use of bacteriocins against *L. monocytogenes*

The production of bacteriocins by lactic acid bacteria has been reported for several decades (Cotter et al., 2005). They are divided into four different classes produced by Gram-positive bacteria (Table 1) (Cotter et al., 2005; Herranz et al., 2001). Some Gram-negative bacteria were also found to be bacteriocinogenic (Oscáriz and Pisabarro, 2001). However, this article only reviews bacteriocins produced by LAB and active against *L. monocytogenes*. Most of these compounds were classified as class II subdivision A bacteriocins, which contain between 37 and 48 amino acids (Fimland et al., 2005). These bacteriocins can be inserted into the bacterial plasmic membrane through its C terminal portion and forms pores that cause the leakage of essential metabolites and loss of the proton motive force, consequently leading to cell death (Balciunas et al., 2013).

Many of these bacteriocins have properties that make them appropriate for utilization as food preservatives. First, they are generally recognized as safe because bacteria that have been present in the human diet for a long period of time produce them. Evidences have shown that they are harmless to eukaryotic cells (Oscáriz and Pisabarro, 2001). Moreover, they are usually resistant to low pH and high temperatures, making them suitable for many processed foods (Herranz et al., 2001). Another advantage of bacteriocins is their relatively easy production in bioreactors at industrial scale. After synthesis, they can be purified and used as food ingredients. Pediocin PA-1 fermentates have been produced and are commercially available as additive to inhibit the growth of spoilage bacteria, including *L. monocytogenes*, in ready-to-eat meats (Rodriguez et al., 2002). However, the introduction of bacteriocins as meat preservatives has been shown to be quite limited despite their promising antimicrobial action when tested *in vitro* (Gálvez et al., 2007). Katla and colleagues (2001) tested the effect of sakacin P (derived from *Lactobacillus sakei*) and nisin on the growth of *L. monocytogenes* in cold smoked salmon. The higher levels of sakacin P tested (1.1 µg/g) could maintain the initial bacterial population for the first 7 days, but *L. monocytogenes* was able to grow and the bacterial counts showed no statistical difference from the control after 28 days. Similar pattern was found for nisin, which could reduce the initial population of *Listeria*, but presented no difference in comparison to the non-treated control products after 21 days. Furthermore, pediciocin AdH produced by either *P. acidilactici* (Goff et al., 1996) or *L. plantarum* (Matilla et al., 2003) was found to inhibit the growth of *L. monocytogenes* in meat, but only a very high dose of the bacteriocin (2,400 AU/g) from *P. acidilactici* were able to cause a ≥3 Log CFU/g reduction of the pathogenic organism.

The main reasons for the decreased antimicrobial activity of bacteriocins in meat products when compared to *in vitro* studies are related to the interaction of these compounds with constituents of the food matrix. Interaction with food phospholipids has been suggested (Leroy and de Vuyst, 1999), as well as the formation of a nisin-glutathione conjugate (Rose et al., 1999). In the latter report, glutathione was found to react with nisin in raw meat by an enzymatic conjugation leading to loss of antimicrobial activity.

Utilization of bacteriocinogenic LAB to control *L. monocytogenes* in dry fermented sausages

Although the utilization of purified bacteriocins to eliminate

Table 1. Classification of bacteriocins produced by Gram-positive bacteria.

Classification	Characteristics	Examples
Class I	Small peptides (<5 kDa) containing the unusual amino acids lanthionine or β-methyl lanthionine.	Nisin, mersacidin, lacticin 481, cytolysin, cinnamycin
Class II	Heterogeneous class of small peptides (<10 kDa), heat-stable. Do not contain lanthionine.	Pediocin, sakacin, leucocin, lactacin A, enterocin, lactococcin, divergicin, carnobacteriocin, reutericin 6
Class III	Large (>30 kDa) heat-labile proteins, often murein hydrolases. Usually possess lytic (enzymatic) activity. Have been reclassified as bacteriolysins.	Helveticin, lysostaphin, enterolysin A, zoocin A, lactacin B
Class IV	Glycoproteins and lipoproteins.	Lactocin 27, sublanicin, glycocin F

L. monocytogenes from dry fermented sausage does not seem to be very promising, *in situ* bacteriocin production has been extensively studied with relative success. Ananou and co-workers (2005) demonstrated that both bacteriocinogenic *E. faecalis* A-48-32 and *E. faecium* S-32-81 were able to reduce the population of *L. monocytogenes* below detection levels in a sausage model after 9 and 6 days of incubation, respectively. The concentration of bacteriocin ranged between 60 and 80 AU/g, which was well below the level of purified bacteriocin needed to cause the same listerial reduction. In addition, bacteriocin-producing *L. curvatus* rapidly (12 h) caused the reduction of *L. monocytogenes* cell counts below detectable levels, while a non-bacteriociogenic commercial starter culture needed 19 days to produce similar results (Benkerroum et al., 2005). Other bacteriocin-producing strains of *Pediococcus* and *Lactobacillus* were also reported to inhibit the growth of *L. monocytogenes* in dry and semi-dry sausages (Abrams et al., 2011; Albano et al., 2007; Hugas et al., 1995).

Interesting results were found by Leroy and colleagues (2005) when testing the effect of bacteriocionogenic *L. sakei* CTC 494 and *L. curvatus* LTH 1174 over the growth of *Listeria innocua* LMG 13568. These researchers simulated the conditions of dry fermented sausage processing in a fermentation bioreactor, and found that both LAB caused a ≥3 Log CFU/g drop on the listerial population. Coincidently, this reduction just started to occur when the production of bacteriocins was activated.

Production of bacteriocins was found to be influenced by a variety of environmental parameters such as temperature, pH, competing bacteria, levels of CO_2, salt, nitrite and others (Gálvez et al., 2007). Therefore, the utilization of a certain bacteriocinogenic LAB as a starter culture will not guarantee the production of bacteriocins in the meat matrix. Apparently, conditions offered to LAB have to meet some requirements in order to achieve high synthesis rates of bacteriocin. Optimum growth conditions were suggested to result to better yield of nisin by *L. lactis*, pediocin AcH by *P. acidilactici*, leuconocin Lcm1 by *Leuconostoc carnosum* Lm1 and sakacin A by *L. sakei* Lb 706 (Yang and Ray, 1994). Conversely, stress caused by moderated doses of salt also stimulated the production of bacteriocin by *Lactobacillus pentosus* B96 (Delgado et al., 2005) and *Lactobacillus amylovorus* DCE 471 (Neysens et al., 2003).

In addition, stress caused by the presence of competing Gram-positive organisms was found to induce the synthesis of plantaricin by *L. plantarum*. Moreover, the bacteriocin itself was also able to promote its own production (Maldonado et al., 2004). More recently, nitrite was found to reduce significantly the production of bacteriocin by *L. curvatus* CWBI-B28 in pork meat (Kouakou et al., 2009), which completely abolished this strain's anti-listerial activity. Therefore, it is extremely important to understand the factors affecting bacteriocin production by a particular strain before its successful application in foods to reduce or eliminate *L. monocytogenes* (Castro et al., 2011).

Minor-Pérez and collaborators (2005) reported that the production of bacteriocin by *L. buchneri* is mainly determined by temperature and atmosphere composition, having 30°C and 100% N_2 as the best choices examined. They also demonstrated that the maximum productivity of bacteriocin occurred during the end of the exponential phase, whereas the production of enterocin by *E. faecium* RZS C5 was found to occur in early stages of the bacterial growth (Leroy and de Vuyst, 2002).

Aymerich and colleagues (2000) examined the influence of additives found in dry fermented sausages on the production of enterocin by *E. faecium* CTC 492. They found that all ingredients tested (salt, nitrate, nitrite and black pepper), with exception of nitrate, reduced the

Table 2. Antimicrobial activity of indigenous bacteriocinogenic LAB used as starter cultures in fermented meats against *L. monocytogenes*.

Organism	*L. monocytogenes* reduction (CFU/g)	Reference
Enterococcus faecalis A-48-32	~3 log	Ananou et al., 2005
Lactobacillus curvatus LBPE	~3 log	Benkerroum et al., 2005
L. sakei CTC 494	≥3 log	Leroy et al., 2005
L. curvatus LTH 1174	≥3 log	Leroy et al., 2005
Pediococcus acidilactici MCH 14	~4 log	Nieto-Lozano et al., 2010
P. acidilactici HA-6111-2	3.2 log	Kingcha et al., 2012
P. pentosaceus BCC 3772	≥3 log	Albano et al., 2009

amounts of enterocin A and B produced. However, natural fermentation was used as the typical method for producing dry fermented sausages for a very long time. Thus, the use of indigenous bacteria present in specific products as their own starter cultures was suggested since they are highly adapted to that environment (Villani et al., 2007) and may generate significant amounts of bacteriocin (Työppönen et al., 2003b).

The utilization of bacteriocinogenic autochthonous bacteria as the starter cultures of dry fermented sausages has been tested (Herranz et al., 2001; Leroy and de Vuyst, 1999; Talon et al., 2008). Table 2 presents several of these organisms that were able to reduce the levels of *L. monocytogenes* in different sausage recipes. These indigenous bacteria survive the rough environment found in these sausages and are able to grow under the manufacture conditions. Selection and identification of these bacteria has been done, and some strains were found to produce high levels of bacteriocin during the fermentation process, offering an extra hurdle against the growth of *L. monocytogenes* (Leroy and de Vuyst, 1999). Albano and colleagues (2007) screened the antimicrobial capacity of 226 LAB strains isolated from a typical naturally fermented Portuguese sausage called ‘‘Alheiras’’. These bacteria were tested against pathogens such as *L. monocytogenes*, *S. aureus*, *E. coli* O157:H7, *Salmonella typhimurium* and *Salmonella enteriditis*. From the initial LAB strains, 14 were found to have antimicrobial effect against *L. monocytogenes*, 4 against *S. aureus*, but none was able to affect the growth of the Gram-negative bacteria. However, only two strains showed antibacterial activity related to bacteriocin production and they were both identified as *P. pentosaceus*. The authors also described that these two strains were able to maintain the levels of *L. innocua* (5 x 10^2 CFU/g) in “Alheiras” sausage paste during 28 days of incubation at 4°C, while *L. innocua* presented a significant outgrowth (1 x 10^7 CFU/g) in the control paste containing a non-bacteriocinogenic strain of *P. pentosaceus*. This bacteriocin was recently identified as bacPPK34 and has shown inhibitory activity against *L. monocytogenes in vitro* (Abrams et al., 2011). Moreover, lactobacilli and pediococci strains isolated from sucuk (typical Turkish fermented sausage) also showed inhibitory activity against *L. monocytogenes*, and the utilization of such LAB as starter cultures specifically for sucuk manufacture was recommended (Çon et al., 2001; Cosansu et al., 2010). A similar study has shown that the use of the autochthonous *P. acidilactici* MCH14, which produces pediocin PA-1, reduces by ~4 log CFU/g the population of *L. monocytogenes* in dry fermented sausage. This reduction was 2-fold higher than what was found for a dry fermented sausage produced with a non-bacteriocinogenic *P. acidilactici* as the starter culture (Nieto-Lozano et al., 2010).

Synthesis of sakacin K by Lactobacillus sakei CTC 494 was found to be temperature and pH-dependent (Leroy and de Vuyst, 1999). This bacterium was isolated from Spanish dry fermented sausage and diverse characteristics of bacteriocin production were evaluated in a model simulating the conditions of the fermenting process. Interestingly, the production of sakacin K was found to achieve the best rates at temperatures between 20 and 25°C and pH 5.0. These values coincide with the typical fermentation temperature and pH of the Spanish sausage. Therefore, it is expected that *L. sakei* CTC 494 might produce high concentrations of sakacin K if used as starter culture of these products. This combination could be highly favourable, since sakacin K was demonstrated to present antilisterial activity (Hugas et al., 1995).

Talon and colleagues (2008) also reported the benefits of using autochthonous LAB as starter cultures in French dry fermented sausage. They isolated, identified and selected three different strains of LAB and examined the microbial, physico-chemical and sensorial aspects of the dry sausage produced. First, the three strains were identified as *L. sakei*, *Staphylococcus equorum* and *Staphylococcus succinus*. Then, dry fermented sausages were crafted using these bacteria as starter cultures and the final products were compared with traditional sausages, which use natural fermentation. The

experimental starter culture showed several advantages over the traditional method. It was found to reduce the levels of harmful biogenic amines, significantly decrease lipid oxidation and maintain the population of *L. monocytogenes* below the levels established by the European Commission Regulation, without sacrificing the sensorial characteristics of the final product. However, the authors did not examine if the pathogen inhibition was related to the production of bacteriocins by the starter culture. Even though, it seems that the utilization of indigenous bacteria as starter cultures can benefit the production of dry fermented sausages, and if well selected, they can generate a safer food with no differences in taste from the traditional sausages. One of the major concerns of using bacteriocinogenic bacteria against *L. monocytogenes* is the resultant pressure for the appearance of bacteriocin-resistant strains. Some listerial strains have already showed lower sensitivity to bacteriocins (Kaur et al., 2011; Martínez et al., 2005). In addition, many bacteriocins present similar structures, and once the resistance to one bacteriocin has been established, it may also confer defense against others.

CONCLUSION

Consumption of dry fermented sausages may present a potential risk to human health due the presence of bacterial pathogens. Even with several barriers against bacterial growth offered by these products, harmful bacteria such as *E. coli* O157:H7 and *L. monocytogenes* were found to persist. Many strategies have been proposed to control the outgrowth of these organisms, such as addition of antimicrobial essential oils (Graumann and Holley, 2008; Luciano et al., 2011) and organic acids (Podolak et al., 1996).

Fermenting bacteria used in dry sausage manufacture, well-known as lactic acid bacteria, produce several hurdles against the growth of pathogenic and spoilage bacteria (organic acids, hydrogen peroxide, diacetyl, etc.). Moreover, these bacteria may produce ribosomally synthesized antimicrobials called bacteriocins. Many of these bacteriocins were found to inhibit the growth of *L. monocytogenes in vitro*, but much higher doses were generally needed to produce the same effect in food products. Selection of bacteriocinogenic strains for utilization as starter cultures of dry fermented sausages was shown to prevent the growth of *L. monocytogenes*, resulting in safer products when compared with non-bacteriocinogenic control strains. Use of autochthonous LAB isolated from fermented sausages has been proposed to offer even better results. Since these bacteria are highly adapted to the food environment, they have a higher chance to outcompete against the growth of pathogenic bacteria. In addition, production of bacteriocins by indigenous bacteria was found to be optimal in the conditions offered by the meat fermentation process. Therefore, their utilization as starter cultures might produce dry fermented sausages with the same sensorial properties of the traditional ones, but presenting a better capacity of inhibiting the growth of *L. monocytogenes*. However, most studies are still very incipient, with many of them using *L. innocua* as a surrogate for *L. monocytogenes* when verifying the antimicrobial activity in dry sausage. Therefore, further studies are necessary to validate that indigenous bacteriocinogenic LAB are capable of eliminating significant levels of *L. monocytogenes*, where several strains of the pathogen must be used to represent genetic variability. Moreover, the bacteriocinogenic starter cultures could be used in combination with other non-thermal technologies, such as high hyperbaric pressure, to guarantee the production of dry sausage free of *Listeria*.

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